Amendments to the Claims

Please amend the claims as follows. This version will replace all prior versions of the claims.

1. (Original) A method of treating HIV-1 infection in a patient by administering a compound that inhibits processing of the viral Gag p25 protein (CA-SP1) to p24 (CA), but has no significant effect on other Gag processing steps.

2. (Original) The method of claim 1 wherein said inhibition does not significantly reduce the quantity of virions released from treated infected cells and/or has no significant effect on the amount of RNA incorporation into the released virions.

3. (Currently amended) The method of claim 1, wherein the compound inhibits the maturation of virions released from treated infected cells.

4. (Original) The method of claim 1, wherein a preponderance of virions released from treated infected cells exhibit spherical, electron-dense cores that are acentric with respect to the viral particle, possess crescent-shaped electron-dense layers lying just inside the viral membrane, and have reduced or no infectivity.

5. (Original) The method of claim 1, wherein the compound inhibits the interaction of HIV protease with CA-SP1, which results in the inhibition of the processing of the viral Gag p25 protein (CA-SP1) to p24 (CA), but has no significant effect on other Gag processing steps.

6. (Original) The method of claim 1, wherein said compound binds to the viral Gag protein such that interaction of HIV protease with CA-SP1 is inhibited.

7. (Original) The method of claim 1, wherein said compound binds near to or at the site of cleavage of the viral Gag p25 protein (CA-SP1) to p24 (CA), thereby inhibiting the interaction of HIV protease with the CA-SP1 cleavage site and resulting in the inhibition of processing of p25 to p24.

8. (Original) The method of claim 1, wherein the HIV infecting said cells does not respond to other HIV therapies.

9. (Original) The method of claim 1, wherein said patient is administered said compound in combination with at least one anti-viral agent.

10. (Original) The method of claim 9, wherein said anti-viral agent is selected from the group consisting of zidovudine, lamivudine, didanosine, zalcitabine, stavudine, abacavir, nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, adefovir, atazanavir, fosamprenavir, hydroxyurea, AL-721, ampligen, butylated hydroxytoluene; polymannoacetate, castanospermine; contracan; creme pharmatex, CS-87, penciclovir, famciclovir, acyclovir, cytofovir, ganciclovir, dextran sulfate, D-penicillamine trisodium phosphonoformate, fusidic acid, HPA-23, eflornithine, nonoxynol, pentamidine isethionate, peptide T, phenytoin, isoniazid, ribavirin, rifabutin, ansamycin, trimetrexate, SK-818, suramin, UA001, enfuvirtide, gp41-derived peptides, antibodies to CD4, soluble CD4, CD4-containing molecules, CD4-IgG2, and combinations thereof.

11. (Original) The method of claim 1, wherein said patient is administered said compound in combination with an immunomodulating agent, anticancer agent, antibacterial agent, antifungal agent, or a combination thereof.

12. (Original) The method of claim 1, wherein said compound is a dimethylsuccinyl betulinic acid or dimethylsuccinyl betulin derivative.

13. (Original) The method of claim 12, wherein said compound is selected from the group consisting of 3-O-(3',3'-dimethylsuccinyl) betulinic acid, 3-O-(3',3'-dimethylsuccinyl) betulin, 3-O-(3',3'-dimethylglutaryl) betulin, 3-O-(3',3'-dimethylsuccinyl) dihydrobetulinic acid, 3-O-(3',3'-dimethylglutaryl) betulinic acid, (3',3'-dimethylglutaryl) dihydrobetulinic acid, 3-O-diglycolyl-betulinic acid, 3-O-diglycolyl-dihydrobetulinic acid and combinations thereof.

14. (Original) The method of claim 13, wherein said patient is administered said compound in combination with at least one anti-viral agent.

15. (Original) The method of claim 14, wherein said anti-viral agent is selected from the group consisting of zidovudine, lamivudine, didanosine, zalcitabine, stavudine, abacavir, nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, adefovir, atazanavir, fosamprenavir, hydroxyurea, AL-721, ampligen, butylated hydroxytoluene; polymannoacetate, castanospermine; contracan; creme pharmatex, CS-87, penciclovir, famciclovir, acyclovir, cytofovir, ganciclovir, dextran sulfate, D-penicillamine trisodium phosphonoformate, fusidic acid, HPA-23, eflornithine, nonoxynol, pentamidine isethionate, peptide T, phenytoin, isoniazid, ribavirin, rifabutin, ansamycin, trimetrexate, SK-818, suramin, UA001, enfuvirtide, gp41-derived peptides, antibodies to CD4, soluble CD4, CD4-containing molecules, CD4-IgG2, and combinations thereof.

16. (Original) The method of claim 13, wherein said patient is administered said compound in combination with an immunomodulating agent, anti-cancer agent, antibacterial agent, an anti-fungal agent, or combinations thereof.

17. (Original) A method of treating human blood products comprising contacting said blood products with a compound that inhibits processing of the viral Gag p25 protein (CA-SP1) to p24 (CA), but has no significant effect on other Gag processing steps.

18. (Original) The method of claim 17 wherein said inhibition does not significantly reduce the quantity of virus released from treated cells and/or has no significant effect on the amount of RNA incorporation into the released virions.

19. (Original) The method of claim 17, wherein the compound inhibits the maturation of virions.released from treated infected cells.

20. (Original) The method of claim 17, wherein the preponderance of said virions released from treated infected cells exhibit spherical, electron-dense cores that are acentric with respect to the virion, possess crescent-shaped electron-dense layers lying just inside the viral membrane, and have reduced or no infectivity.

21. (Original) The method of claim 17, wherein the compound inhibits the interaction of HIV protease with CA-SP1, which results in the inhibition of the processing of the viral Gag p25 protein (CA-SP1) to p24 (CA), but has no significant effect on other Gag processing steps.

22. (Original) The method of claim 17, wherein said compound binds near to or at the site of cleavage of the viral Gag p25 protein (CA-SP1) to p24 (CA), thereby inhibiting the interaction of HIV protease with CA-SP1 and resulting in the inhibition of processing of p25 to p24.

23. (Original) A method for identifying compounds that inhibit HIV-1 replication in cells of an animal, comprising:

(a) contacting a Gag protein comprising a CA-SP1 cleavage site with a test compound;

(b) adding a labeled substance that selectively binds at or near the CA-SP1 cleavage site; and

(c) measuring competition between the binding of the test compound and the labeled substance to the CA-SP1 cleavage site.

24. (Original) The method of claim 23, wherein the compounds inhibit the interaction of HIV-1 protease with a target site by binding to said target site.

25. (Original) The method of claim 23, wherein the CA-SP1 is contained within a polypeptide fragment or recombinant peptide.

26. (Original) The method of claim 23, wherein the labeled substance is a labeled antibody specific for CA-SP1, and measuring the change in the amount of labeled antibody bound to the protein in the presence of test compound compared with a control.

27. (Original) The method of claim 23, comprising measuring the change in the amount of labeled 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid bound to the protein in

the presence of test compound, compared with a control, and wherein the labeled substance is 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid.

28. (Original) The method according to claim 23 wherein the label is selected from the group consisting of an enzyme, fluorescent substance, chemiluminescent substance, horseradish peroxidase, alkaline phosphatase, biotin, avidin, electron dense substance, radioisotope and a combination thereof.

29. (Original) A method for identifying compounds that inhibit HIV-1 replication in the cells of an animal comprising:

(d) contacting a Gag protein comprising a wild-type CA-SP1 cleavage site, with HIV-1 protease in the presence of a test compound;

(e) separately, contacting a Gag protein comprising a mutant CA-SP1 cleavage site or a protein comprising a alternative protease cleavage site with HIV-1 protease in the presence of the test compound; and

(f) comparing the amount of cleavage of the native wild-type Gag protein to the amount of cleavage of the mutant Gag protein or to amount of cleavage of the protein comprising an alternative protease cleavage site.

30. (Original) The method of claim 29, wherein the wild-type CA-SP1 or mutant CA-SP1 or alternative protease cleavage site is contained within a polypeptide fragment or recombinant peptide.

31. (Original) The method of claim 29, wherein said Gag protein is labeled with a fluorescent moiety and a fluorescence quenching moiety, each bound to opposite sides of the CA-SP1 cleavage site, and wherein said detecting comprises measuring the signal from the fluorescent moiety.

32. (Original) The method of claim 29, wherein said Gag protein is labeled with two fluorescent moieties, each bound to opposite sides of the CA-SP1 cleavage site, and wherein said detecting comprises measuring the transfer of fluorescent energy from one moiety to the other in the presence of the test compound.

33. (Original) The method of claim 29 wherein the effect of the test compound on cleavage of the Gag protein is detected by measuring the amount of a labeled antibody that is bound to SP1 or p24 (CA).

34. (Original) The method of claim 33, wherein the labeled antibody that binds CA, or the antibody that binds SP1 is labeled with a molecule selected from the group consisting of enzyme, fluorescent substance, chemiluminescent substance, horseradish peroxidase, alkaline phosphatase, biotin, avidin, electron dense substance, radioisotope, and combinations thereof.

35. (Original) A method for identifying compounds that inhibit HIV-1 replication in cells of an animal comprising:

(g) contacting a test compound with wild-type virus isolates and separately with virus isolates resistant to 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid; and

(h) selecting test compounds that are more active against the wild-type virus isolate compared with virus isolates that are resistant to 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid.

36. (Original) A method for identifying compounds that inhibit HIV replication in the cells of an animal, comprising:

(i) contacting HIV-1 infected cells with a test compound; and

(j) thereafter lysing the infected cells or the released viral particles to form a lysate, and analyzing the lysate to determine whether cleavage of the CA-SP1 protein has occurred.

37. (Original) The method of claim 36, wherein said analyzing comprises measuring the presence or absence of p25.

38. (Original) The method of claim 36, wherein said analyzing comprises performing a western blot of viral proteins and detecting p25 using an antibody to p25.

39. (Original) The method of claim 36, wherein said analyzing comprises performing a gel electrophoresis of viral proteins and imaging of metabolically labeled proteins.

40. (Original) The method of claim 36, wherein said analyzing comprises using an antibody that selectively binds cleaved p24 (CA) or SP1 to distinguish p25 from p24.

41. (Original) A method for identifying compounds that inhibit HIV-1 replication in the cells of an animal comprising contacting HIV-1 infected cells with a test compound and thereafter analyzing, wherein the virus particles released by the cells are analyzed by using transmission electron microscopy, for the presence of spherical cores that are acentric with respect to the viral particle, and having crescent-shaped, electron-dense layers lying just inside the viral membrane.

42. (Original) An isolated polynucleotide comprising a sequence which encodes an amino acid sequence containing a mutation in an HIV Gag p25 protein (CA

SP1), said mutation resulting in a decrease in inhibition of processing of p25 (CA-SP1) to p24 (CA) by 3-O-(3',3'-dimethylsuccinyl) betulinic acid.

43. (Original) The isolated polynucleotide of claim 42, wherein said decrease in inhibition of processing of p25 is due to a decrease in inhibition of the interaction of HIV-1 protease with Gag.

44. (Original) The isolated polynucleotide of claim 42, wherein said decrease in inhibition of processing of p25 is due to a decrease in the binding of 3-O-(3',3'-dimethylsuccinyl) betulinic acid to Gag.

45. (Original) The isolated polynucleotide of claim 42, wherein said decrease in inhibition of processing of p25 is due to a decrease in the binding of DSB at or near the CA-SP1 cleavage site of Gag.

46. (Original) The isolated polynucleotide of claim 42, wherein said mutation is located in the SP1 region of CA-SP1.

47. (Currently amended) The isolated polynucleotide of claim 42, wherein said mutation is located in the amino acid sequence ~~KARVL/IAEAMS~~ KARV/ILAEAMS (SEQ ID NO: 1).

48. (Currently amended) The isolated polynucleotide of claim 42, wherein said mutation comprises an amino acid sequence that is selected from the group consisting of KARVLVEAMS (SEQ ID NO: 2) or ~~KARVIAEVMS~~ KARILAEVMS (SEQ ID NO: 3).

49. (Original) The isolated polynucleotide of claim 42, comprising an amino acid sequence encoded by a polynucleotide which is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 9.

50. (Original) The isolated polynucleotide of claim 42, having 95% identity to a polynucleotide selected from the group consisting of SEQ ID NO: 4, and SEQ ID NO: 6.

51. (Original) The isolated polynucleotide of claim 42, having 80% identity to a polynucleotide selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 9.

52. (Original) The isolated polynucleotide of claim 42, having 95% identity to a polynucleotide selected from the group consisting of SEQ NO: 5 and SEQ ID NO: 7.

53. (Original) The isolated polynucleotide of claim 42, having 80% identity to a polynucleotide of SEQ ID NO: 10.

54. (Original) A vector comprising the isolated polynucleotide of claim 42.

55. (Original) A host cell comprising the vector of claim 54.

56. (Original) A method of producing a polypeptide comprising incubating the host cell of claim 55 in a medium and recovering the polypeptide from said medium.

57. (Original) A virus comprising the isolated polynucleotide of claim 42.

58. (Original) A retrovirus comprising the isolated polynucleotide of claim 42.

59. (Original) The retrovirus of claim 58, selected from the group consisting of HIV-1, HIV-2, HTLV-I, HTLV-II, SIV, avian leukosis virus (ALV), endogenous avian retrovirus (EAV), mouse mammary tumor virus (MMTV), feline immunodeficiency virus (FIV), or feline leukemia virus (FeLV).

60. (Original) The retrovirus of claim 59 which is HIV-1.

61. (Original) A polypeptide containing a mutation in an HIV CA-SP1 protein, said mutation which results in a decrease in inhibition of processing of p25 by 3-O-(3',3'-dimethylsuccinyl) betulinic acid.

62. (Original) The polypeptide of claim 61, wherein said mutation is located in the SP1 region of SEQ ID NO: 5, SEQ ID NO: 7, or SEQ ID NO: 10.

63. (Original) The polypeptide of claim 61, which is encoded by a polynucleotide selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 9.

64. (Currently amended) The polypeptide of claim 61, wherein said mutation comprises a sequence that is selected from the group consisting of KARVLVEAMS (SEQ ID NO: 2) or ~~KARVIAEVMS~~ KARILAEVMS (SEQ ID NO: 3).

65. (Original) The polypeptide of claim 61, encoded by an isolated polynucleotide which hybridizes under highly stringent conditions to a polynucleotide selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 7, and 10.

66. (Original) The polypeptide of claim 61, wherein said polypeptide is part of a chimeric or fusion protein.

67. (Original) An antibody which selectively binds an amino acid sequence containing a mutation in an HIV CA-SP1 protein which results in a decrease in the inhibition of processing of p25 (CA-SP1) to p24 (CA) by 3-O-(3'3'-dimethylsuccinyl) betulinic acid.

68. (Original) The antibody of claim 67, wherein said mutation is located in the SP1 region of CA-SP1.

69. (Currently amended) The antibody of claim 68, wherein said mutation comprises a sequence that is selected from the group consisting of KARVLVEAMS (SEQ ID NO: 2) or ~~KARVIAEVMS~~ KARILAEVMS (SEQ ID NO: 3).

70. (Original) The antibody of claim 67, which selectively binds an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 3.

71. (Original) An antibody that selectively binds SP1 but not CA-SP1.

72. (Original) An antibody that selectively binds CA but not CA-SP1.

73. (Original) An antibody that selectively binds at or near the CA-SP1 cleavage site.

74. (Original) A compound identified by the method of claim 23, 29, 35, 36, or 41, wherein the compound is not a compound selected from the group consisting of 3-O-(3',3'-dimethylsuccinyl) betulinic acid, 3-O-(3',3'-dimethylsuccinyl) betulin, 3-O-(3',3'-dimethylglutaryl) betulin, 3-O-(3',3'-dimethylsuccinyl) dihydrobetulinic acid, 3-O-(3',3'-dimethylglutaryl) betulinic acid, (3',3'-dimethylglutaryl) dihydrobetulinic acid, 3-O-diglycolyl-betulinic acid, 3-O-diglycolyl-dihydrobetulinic acid, and combinations thereof.

75. (Original) A pharmaceutical composition comprising one or more compounds according to claim 74, or a pharmaceutically acceptable salt, ester or prodrug thereof, and a pharmaceutically acceptable carrier.

76. (Original) A pharmaceutical composition comprising a compound identified by the method of claim, 23, 29, 35, 36, or 41, said composition further comprising an anti-viral agent.

77. (Original) The pharmaceutical composition of claim 76 which comprises a dimethylsuccinyl betulinic acid or dimethylsuccinyl betulin derivative.

78. (Original) The pharmaceutical composition of claim 76, wherein said compound is selected from the group consisting of 3-O-(3',3'-dimethylsuccinyl) betulinic acid, 3-O-(3',3'-dimethylsuccinyl) betulin, 3-O-(3',3'-dimethylglutaryl) betulin, 3-O-(3',3'-dimethylsuccinyl) dihydrobetulinic acid, 3-O-(3',3'-dimethylglutaryl) betulinic acid, (3',3'-dimethylglutaryl) dihydrobetulinic acid, 3-O-diglycolyl-betulinic acid, 3-O-diglycolyl-dihydrobetulinic acid, and combinations thereof.

79. (Original) The pharmaceutical composition of claim 76, wherein said antiviral agent is selected from the group consisting of zidovudine, lamivudine, didanosine, zalcitabine, stavudine, abacavir, nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, adefovir, atazanavir, hydroxyurea, AL-721, ampligen, butylated hydroxytoluene; polymannoacetate, castanospermine; contracan; creme pharmatex, CS-87, penciclovir, famciclovir, acyclovir, cytofovir, ganciclovir, dextran sulfate, D-penicillamine trisodium phosphonoformate, fusidic acid, HPA-23, eflornithine, nonoxynol, pentamidine isethionate, peptide T, phenytoin, isoniazid,. ribavirin, rifabutin, ansamycin, trimetrexate, SK-818, suramin, UA001, and combinations thereof.

80. (Original) The pharmaceutical composition of claim 76, further comprising an immunomodulating agent, an anti-cancer agent, an anti-fungal agent, an anti-bacterial agent, or combinations thereof.

81. (Original) A method of determining if an individual is infected with HIV-1 that is susceptible to treatment by a compound that inhibits p25 processing that involves taking blood from the patient, genotyping the viral RNA and determining whether the viral RNA contains mutations in the sequence encoding the region of the CA-SP1.